REMARKS

A check for the fee for a three month extension of time accompanies this response. Any fee that may be due in connection with this application may be charged to Deposit Account No. Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 22-25, 28, 29, 31-33, 155-158, 164-168 and 170-172, 211, 212, 213, 216 and 217 are pending. Claims 1, 4, 6, 8-13, 15, 26, 27, 30, 34, 35, 159-161, 163, 169, 173-210, 214 and 215 are cancelled without prejudice or disclaimer as being allegedly withdrawn from consideration as being drawn to non-elected subject matter. Applicant reserves the right to file divisional applications to the withdrawn subject matter; the Office is reminded that as between any of the cancelled claims and the presently pending claims obviousness-type double patenting cannot be held.

Claim 157 is indicated as being drawn to non-elected subject matter. Applicant does not understand the basis for this; hence claim 157, which is dependent on claim 155 is retained. If claim 155 is patentable, claim 157 must be patentable, since a dependent claim includes all limitations of the base claim.

Claim 211, which is indicated as being drawn to non-elected subject matter, remains pending. Claim 211 is **not** restrictable from claim 155, since it the subject matter is overlapping., Also, claim 211 is claim 2 from the parent application, and, hence, unequivocally finds basis in that case. Furthermore, it reads on the elected subject matter, and, hence is retained.

Claims 212, 213, 215 and 216 are also retained. Claims 212 and 213 are dependent on claim 211; and claims 215 and 216 are dependent upon claim 22.

A marked up copy of claims showing the amendments herein is appended hereto.

BASIS IN PARENT APPLICATION

It is respectfully submitted that pending claims do indeed find basis in the parent application. As discussed previously, the test for new matter is that the specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). The specification as originally filed conveys with reasonable clarity disclosure that supports all of the pending claims and the claims of this application as originally filed.

In the parent application, U.S. provisional application Serial No. 60/044,693 (converted to a provisional from application Serial No. 08/506,608) claim 1 recites:

> 1. A method for generating autologous effector immune cells, the method comprising:
>
> collecting material leukocyte containing material from a mammal; and
> exposing the leukocyte containing material to mitogenic monoclonal antibodies to induce *in vitro* cell proliferation sufficient for infusion into the mammal for use in an immunotherapy treatment, wherein the *in vitro* cell proliferation is produced without the use of exogenous interleukin-2.

As an aside, because of a restriction requirement claim 1 is now pending one of the co-pending divisional applications. In that case, we will demonstrate actual reduction to practice before the effective filing date of the U.S. application that corresponds to the June *et al.* PCT application at issue in the instant case.

Claim 2 of the parent application recites:

> 2. The method of claim 1, wherein the leukocyte containing material is caused to differentiate into desired effector cells.

Hence claim 2 corresponds to presently pending claim 211, which has been amended to recite Th1 cells (see, claim 7 in the parent application, which includes Th1 and Th2 cells as species of effector cells, under the old definition).

Claim 14 recites that the cells are proliferated to "an excess of 1×10^{10} cells."

Claim 7 recites that the cells are Th1-like or Th2-like cells, thereby indicating that as originally filed, what are now called regulatory cells were contemplated to be within the scope of the original claims and to be separately claimed.

The parent application is directed to methods for the production of high concentrations and amounts of homogeneous compositions of immune cells, including Th1, Th2, and also LAK, CTL and TIL cells. In the parent application, the term "effector" cell was used to encompass all types. Dependent claims separated out the Th1 and Th2 cells from the generic type. Claim 1 generically encompassed all types of cells.

In the instant application, the nomenclature, **not** the intended scope of the claims, was modified so that the generic language refers to what was called effector cells in the parent application as "immune cells" Compare claim 1 as originally in the instant case with claim 1 of the parent case. The language "effector" is changed to immune cell. Further, two classes of cells were defined: regulatory immune cells, which are clearly defined (as discussed below) to include Th1 and Th2 cells, which can be identified by their distinct cytokine profiles and which act on other cells; and effector immune cells, which are defined as the LAK and TIL type cells.

The parent specification states at page 7, line 16. that effector cells include Th1, Th2-like cells. The specification describes Th1 and Th2 cells at page 8, lines 27, page 9, line 3, and page 9, lines 20-24; and states at page 9, lines 25-28:

> Accordingly, it is desirable to have the ability to produce large quantities of autologous Th1 T-cells in disease states where a Th2 cytokine profile predominates (infectious disease) and Th2 T-cells in a TH1-dominant disease (chronic inflammation and autoimmune disease).

Methods for differentiation of immune cells into Th1 or Th2 cells are described at page 11, lines 11-19.

The scope of the claims and subject matter of the claims as originally filed and as filed in the instant cased are the same. The definitions were slightly modified in the instant case; but it is clear from the context that the broad claims and dependent claims are the same.

The parent application states, starting at page 6, line 23, that:

> use of ACI protocols will require technology that enables: the generation of homogeneous populations of immune effector cells [*i.e.*, cells that include TILs, LAKs, CTLs, Th1, Th2 cells]; the consistent growth of effector cells to clinically relevant dosages (*i.e.*, greater than 10^{10} cells) without the use of IL-2; . . . and the ability to reinfuse the cells without the need for systemic infusion of IL-2. Furthermore additional *in vitro* differentiation strategies are need to broaden the types of cells available for ACI protocols.

The present invention addresses each of these requirements, disclosing a method to differentiate Th1 or TH2 cells *in vitro* and grow these cells to clinically relevant numbers without the use of IL-2.

This provides clear unequivocal basis for claim 1 in the present application.

The instant application has been rewritten for clarity, not to add new matter to the claims, and to provide additional supporting examples. To distinguish between differentiation of cells to produce LAKs, TILs and CTLs, from differentiation to produce Th1, Th2 and Th3 cells and subcategories thereof, different nomenclature has been adopted. The same cells are encompassed by the claims; their names are different. Changing the names of claimed subject matter does not add new matter if the substance remains substantially the same.

Similarly, claim 155 and 197 find basis in the parent application. Claim 155 recites:

> A method for generating clinically relevant numbers of regulatory T lymphoid cells for autologous cell therapy, comprising:
> (a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;
> (b) treating the cells to induce differentiation of mononuclear cells into regulatory T cells, wherein regulatory T cells are mononuclear cell

that have the ability to control or direct an immune response, but do not act directly as effector cells in the response; and

(c) contacting the resulting differentiated cells with one or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory cells for autologous cell therapy are generated.

Basis for claim 155 may be found in original claim 2, which includes the steps of obtaining the cells (see, also page 10, lines 15-20, which describes the step of obtaining mononuclear cells), causing them to differentiate (see, also page 10, line 25, - page 11, line 21 which describes means to cause differentiation of the collected cells into various cell types, including using Il-2) and expanding the differentiated cells. Basis for claim 197 may be found in original claim 2.

In the interest of advancing prosecution (not to alter the scope or content of the claims), the word "regulatory" or "effector" no longer appears in the claims, thereby rendering the issue with respect to nomenclature moot. The word regulatory is not needed, since the claims now specifically recite Th1 cells in order to conform the case to the elected subject matter. Conforming claims to a requirement for election **cannot then result in an assertion by the Patent Office that such amendment adds new matter**; if it does, then the requirement for election is improper.

Furthermore, by reciting the names of the cells rather then their functional definition, it does not matter whether they are called effector cells (under older art definitions) or regulatory cells under current definitions. They are the same cells and would be the same cells (*i.e.* Th1 cells), if the were called "Fred or Harry." Th1 cells are the among the cells that can are produced by the methods herein.

Notwithstanding the fact that rejection is moot, points raised by the Examiner are addressed. Before addressing them, it is first noted that the

Examiner must provide scientific basis for his objections. Th Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . ..

The Examiner is urging that one of skill in the art would not understand the definition of "regulatory cells" and "effector cells" and provides no scientific basis for these conclusions. The specification unambiguously defines these terms.

The Examiner urges, without basis, that the definition of regulatory immune cells is unclear. As noted previously, the instant specification (page 19) clearly defines a regulatory cell to encompass any mononuclear cell with a defined cytokine production profile in which such cytokine profile does not directly mediate an effector function" and that the cell has the ability to control or direct an immune response, but does not act as an effector cell in the response. Th1, Th2, Th3 and other helper T cells fall within this definition. The specification states:

> As used herein, a regulatory immune cell is any mononuclear cell with a defined cytokine production profile and in which such cytokine profile does not directly mediate an effector function. A regulatory immune cell is a mononuclear cell that has the ability to control or direct an immune response, but does not act as an effector cell in the response. Regulatory immune cells exert their regulatory function by virtue of the cytokines they produce and can be classified by virtue of their cytokine production profile. For example, regulatory immune cells that produce IL-2 and IFN-γ, but do not produce IL-4 are termed "Th1" cells. Regulatory immune cells that produce IL-4 and IL-10, but do not produce IFN-γ are termed "Th2" cells. Regulatory immune cells that produce TGF-β, IL-10 and IFN-γ, but do not produce IL-2 or IL-4 are termed "Th3" cells. Cells that produce Th1, Th2 and Th3 cytokine profiles occur in CD4+ and CD8+ cell populations. Cells that produce IL-2, IL-4 and IFN-γ are thought to be precursors of Th1 and Th2 cells and are designated "Th0" cells. Populations of cells that produce a majority of Th1 cytokines are designated "Th1-like"; populations

> producing a majority of the Th2 cytokines are designated Th2-like"; those producing a majority of Th3 cytokines are designated "Th3-like". Thus, each composition, although containing a heterogeneous population of cells, will have the properties that are substantially similar, with respect to cytokine, to the particular Th subset.
>
> It is understood that this list of T- cells is exemplary only, and any other definable population, array or subtype of T cells that can be expanded by the methods herein to clinically relevant numbers are intended herein.

Effector cells are defined to be LAK, TIL, CTL and B cells. At page 20, the specification states:

> As used herein, effector cells are mononuclear cells that have the ability to directly eliminate pathogens or tumor cells. Such cells include, but are not limited to, LAK cells, MAK cells and other mononuclear phagocytes, TILs, CTLs and antibody-producing B cells and other such cells.

The Examiner further urges that the art recognizes that Th1 cells are effector cells in autoimmune diseases, citing the fact that Th1 cells have been shown to be involved in the pathogenesis of diabetes and that the Th1 cells are activated upon recognition of islet antigens.

First, the Examiner misunderstands the role of Th1 cells and their cytokines. They <u>do not</u> act as effectors in mediating islet destruction. In accord with the definitions in the instant case, they produce cytokines that ***regulate*** and ***direct*** the islet destruction by CD8+ CTL effector cells. Effector cells are able to mediate lysis of only cognate target cells presenting relevant peptides in the appropriate major histocompatibility complex molecule. Direct effector cell mediated cytotoxicity occurs through the release of perforin and granzymes. CD4+ CTL have been described that are able to directly lyse cells through cognate interaction by producing perforin. These CTL cells are not within the scope of the definition of regulatory cells in the instant case, but fall within the definition of effector cells; the instant case clearly states that CTL cells are effector cells.

Second, as noted, it does not matter what definition th Th1, Th2, etc. cells fall within, there is no evidence of record that one of skill in the art does not know what at helper T cells is nor what particular subsets, such as Th1, are.

The Examiner, without any basis for his statement, further urges that the art recognizes that lymphotoxin can be directly cytotoxic to tumor cells and that Th1 cells produce lymphotoxin, concluding that Th1 cells would not be regulatory cells under our definition because a Th1 cytokine has a direct cytotoxic effect. This is incorrect, since the Th1 cell is not directly mediating the effect, but releases a lymphotoxin, which allegedly mediates the effect.

Again the Examiner, again failing to cite any art to support his statement, is further mistaken that lymphotoxin has a direct cytolytic effect. Lymphotoxin, which is related to TNF, mediates its effect on cells through the binding of high affinity TNF transmembrane receptors. It is not direct lysis like that caused by perforin and granzyme. The cytolytic mechanism initiated by lymphotoxin is indirect through receptor signaling leading to an apoptotic form of cell death. Therefore, Th1 cells that produce lymphotoxin are regulatory cells by our definition.

Again without basis, the Examiner also states that "additional confusion" comes from the fact that we refer to B cells as effector cells and not regulatory cells. He argues that B cells, because they do not kill target cells by a contact mediated mechanism would be regulatory cells under our definition. Again the Examiner is mistaken.

First, any skilled artisan, knows that B cells are not regulatory. Second, the instant specification specifically states that B cells are effector cells. Further, B cells do not fall within the definition of regulatory cells, since they do not mediate their effects via the cytokines expressed. B cells do not have a defined cytokine profile and do not control or direct an immune response, they are not regulatory cells under the definition in the instant specification.

It is very clear from the specification and the definitions therein that a regulatory cell is a cell that exerts its regulatory function by virtue of the cytokines produced and can be classified by virtue of their cytokine production profile. Hence Th1 and Th2 cells are regulatory cells.

Effector cells on the other hand exert their activity by directly eliminating pathogens or tumor cells. Such cells include, but are not limited to, LAK cells, MAK cells and other mononuclear phagocytes, TILs, CTLs and antibody-producing B cells and other such cells.

It is also eminently clear, that in the parent application the term effector cells was used more broadly in accord with its original meaning and as described in the specification encompassed Th1 cells. As discussed previously and above, more recent art has divided what were previously called effector cells into effector cells and regulatory cells.

There can be no confusion with respect to the meaning in the instant cases since the specification specifically states that Th1 cells are under new nomenclature regulatory cells. The claims now recite that the cells are Th1 cells. Hence the identity of the cells produced by the claimed methods is unequivocal.

THE REJECTION OF CLAIMS 22-29 31-35, 155, 156, 158, 160, 162, 164-168 AND 170-172 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

It is respectfully submitted that the grounds for this rejection are rendered moot. As discussed above, all claims are amended to recite that the cells are Th1 cells to conform to the election requirements. Hence, the language to which the Examiner objects is not present in the claims. Whether the are called regulatory or effector cells, they are Th1 cells and are defined by their cytokine profile. All claims are directed to methods for producing Th1 cells.

CLAIM FOR PRIORITY

As discussed above and previously, Applicant disagrees with the Examiner's contention that instantly claimed subject matter does not find basis in the parent application. As discussed in great detail in previous responses and above, the parent application described methods for production of Th1 and Th2

cells, by obtaining l ukocyte-containing material, optionally subjecting the leukocytes to conditions, such as exposure to a set of cytokines, to direct differentiation into Th1 or Th2 cells, and then exposing the cells to mitogenic signals, in the absence of Il-2, and expanding the cells to clinically relevant numbers of cells. This case and the parent case describe the same subject matter. Therefore the claims, as discussed previously, find basis in the parent application.

THE REJECTION OF CLAIMS 22-28, 34, 155, 156, 158, 160, 162, 164, 167, 168 AND 170-172 UNDER 35 U.S.C. § 102(e)

Claims 22-28, 34, 155, 156, 158, 160, 162, 164, 167, 168 and 170-172 are rejected under 35 U.S.C. § 102(e) as being anticipated by Babbitt *et al.* (U.S. Patent No. 5,766,920) because Babbitt *et al.* allegedly discloses a method for production of Th1 cells. This rejection is respectfully traversed.

Relevant law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S.. 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally

from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

Claims

Claim 22 is directed to a method for generating clinically relevant cell numbers of Th1 cells. The steps of the method include:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the T lymphoid cells to alter their cytokine production profile by causing differentiation of the cells into Th1 cells; and

(c) in the absence of IL-2, inducing cell proliferation and expanding the cells under conditions that produce at least about 10^{10} cells/liter of a homogeneous population of Th1 cells, wherein a homogeneous population of Th1 cells comprises greater than about 50% Th1 cells.

Dependent claims specify particulars, including the volume of the resulting population of cells.

Claim 155 is also directed to a method for generating clinically relevant numbers of Th1 cells by:

(a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

(b) treating the cells to induce differentiation of mononuclear cells into Th1 cells; and

(c) contacting the resulting differentiated cells with two or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of cells for autologous cell therapy are generated, wherein the contacting is effected in the absence of exogenous cytokines.

Claim 211 is directed to o a method for generating clinically relevant numbers of Th1 cells by:

collecting leukocyte containing material from a mammal;
differentiating the leukocytes into Th1 cells;
and
exposing the leukocyte containing material to two or more mitogenic monoclonal antibodies to induce *in vitro* cell proliferation sufficient for infusion into the mammal for use in an immunotherapy

> treatment, wherein the *in vitro* cell proliferation is produced without the use of exogenous interleukin-2.

Hence in claim 155, the expansion step is specifically effected in the absence of any exogenous cytokines; in claims 22 and 211, the expansion step is effected in the absence of Il-2. In claim 211, the expansion is effected by two mitogenic monoclonal antibodies; and in claim 155 by two proteins specific for cell surface molecules.

Babbitt *et al.* and differences from the instantly claimed methods

> Babbitt discloses a method for production of "immunoreactive cells" by:
>
> (a) contacting a sample of mononuclear cells derived from a patient, e.g., peripheral blood mononuclear cells (PBMC), with OKT3 at or below 37° C to produce an OKT3-derived culture supernatant (T3CS);
> (b) removing the T3CS from the sample of patient- derived mononuclear cells;
> (c) determining the concentration of OKT3 in the T3CS, and if required, supplementing the T3CS with additional OKT3 to achieve a concentration of at least 0.1 ng/ml;
> (d) providing a second sample of mononuclear cells derived from the patient; and,
> (e) contacting the second sample of cells with the previously-generated T3CS for a period of time sufficient to yield a population of immunoreactive cells.
>
> The "immunoreactive cells are described by Babbitt as **polyclonal T-cells that exist in a primed state of activation**:
>
> are multifunctional, i.e., possess an enhanced capacity to proliferate and produce cytokines ***upon further stimulation.*** (emphasis added)

These immunoreactive cells are stated to have a:

> low spontaneous level of immune function following processing, but are highly sensitized to respond to *low doses of second signals* up on further culture or *in vivo*.(emphasis added)

At column 4, lines 6-17, Babbitt states:

> Immunoreactive cells have a low spontaneous level of immune function following processing, but are highly sensitized to respond to low doses of second signals upon further culture, or in vivo. The immunoreactive cells of the invention **therefore requir furth r xposur to an immune stimulant,** such as an antigen; target cell, e.g., a tumor cell or

> virus-infected cell; an inflammatory molecule; an adhesion molecule; an immune cell, e.g., an accessory cell; a cytokine; or any combination thereof, **to achieve full immunologic effector function.** The immunoreactive cells of the invention are multifunctional, polyclonally-activated T cells which have been generated independent of disease-specific antigens utilizing a mixture of nonspecific lymphocyte activators, i.e., autologous cytokines, and a mouse monoclonal antibody, i.e, OKT3, as synergistic stimulants.

Babbitt continues:

> The ability of EVA cells [the immunoreactive cells] to proliferate and to produce a variety of cytokines (IL-2, GM-CSF, IFN.gamma., TNF.alpha.) in vitro in response to further stimulation by such agents as PMA and IL-2, as well as to lyse tumor cell targets, is greatly enhanced compared to the PBMC from which they were derived. The lowered activation threshold of the EVA cells exhibited in vitro suggests that once they are reinfused into patients, they are likely to demonstrate enhanced responsiveness to immunological signals, such as weakly immunogenic tumor antigens which normally are non-stimulatory to unprocessed cells.

Hence the immunoreactive cells produced by Babbitt are not Th1 cells nor does Babbitt disclose or suggest that they are Th1 cells. The immunoreactive cells, designated EVA cells, are cells that have low spontaneous levels of immune function following processing and require further signals to function. The Babbitt cells, as stated in Babbitt "display very little spontaneous proliferation or cytokine secretion without PMA stimulation". As stated in Babbitt (see above), the cells must have exogenous IL-2 or other stimulation to **achieve full immunologic effector function.**

> Furthermore, Babbitt states that the:
>
> T cells which have been polyclonally-activated independent of tumor-associated antigens according to the invention, possessed a greatly enhanced cytotoxicity toward both tumor lines compared to unprocessed PBMC. These in vitro results suggest that EVA cells are capable of directly killing tumor cells in vivo.

Hence the cells produced by the possess effector function, nor regulatory function as defined in the instant application. he Babbitt cells are capable of "directly killing tumor cells in vivo" . Thus they ar not regulatory cells as

defined in the instant application nor are they disclosed or sugg sted to be Th1 cells as understood by the art.

The discussion in Babbitt directed to "Th1" cells refers to the production of the cells used to produce the T3CS supernatant. These cells are discarded, not expanded. The T3CS supernatant is then used to as part of the culture medium for additional cells, and the resulting cells are designated immunoreactive cells, which as discussed above, are not Th1 cells nor does Babbitt suggest they are.

In order to generate Th1 cells, as described in the instant application, the cytokine environment is controlled at the time of activation in order to achieve optimal regulatory cell differentiation. The Babbitt method uses a cytokine soup that is uncontrolled and varies from patient to patient. Babbitt simply polyclonally activates a patient's PBMC and collects the supernatant. This is a random uncontrolled process. The supernatant is harvested and subsequently used.

The Examiner urges that the Babbitt cells are treated by one or more activating proteins. The fact is that the Babbitt cells are only contacted by one activating protein, OKT3. The Examiner also urges that the Babbitt cells are purified from the material, when in fact the Babbitt process has no purification step whatsoever. The specification states that the starting material is a mixture of T cells, B cells, monocytes and macrophages as well as other immune cells. The Babbitt cells most preferably have 10% monocytes.

Analysis

Anticipation requires disclosure of every element as claimed in the cited reference. Babbitt fails to disclose a step of differentiating the cells into Th1 cells, and then expanding the cells in the absence of exogenous cytokines, including IL-2.

Babbitt *et al.* is directed to a method for producing immunoreactive cells by contacting a first sample of mononuclear cells with OKT3 at or below 37° C

to "produce an OKT-3-derived culture supernatant (T3CS)", removing th T3CS from the mononuclear cells; optionally supplementing the T3CS with additional OKT3 to produce a concentration of at least 0.1 ng/ml of OKT3, and then contacting a second sample of mononuclear cells with the T3CS to produce immunoreactive cells, which Babbitt states require further treatment, such as exposure to Il-2 for activation.

To produce that T3CS is conditioned medium Babbitt only adds a single monoclonal antibody, OKT3. Babbitt does not disclose or suggest activating with two or more proteins, Hence Babbitt *et al.* is directed to a method for producing a supernatant that is used to produce cells that can be activated by subsequent exposure to exogenous cytokines. Any teachings disclosure of expansion of the cells contemplates using IL-2 to effect expansion (see column 18, lines 7 -20). As exemplified, expansion only produces 10^9 cells, not the 10^{10} cells contemplated in the instant application. Thus, Babbitt *et al.* does not teach a method for producing clinically relevant numbers of cells in which expansion is effected in the absence of exogenous IL-2. Babbitt *et al.* does not teach or suggest a step of expanding the cells to clinically relevant numbers in the absence of IL-2.

The method of Babbitt *et al.* does not use mitogenic antibodies for activation, but rather uses a supernatant and a single monoclonal antibody. Furthermore, Babbitt *et al.* does not suggest the step of expanding the selected cells clinically relevant numbers in the absence of interleukin-2 (IL-2).

Thus, Babbitt discloses a method involving obtaining cells from a patient, treating the cells with a single mitogenic factor OKT3, and optionally adding exogenous cytokines (see, col. 5, lines 2-15), collecting the supernatant from such cells, and contacting the supernatant with additional cells from a patient to produce "immunoreactive cells" that require further stimulation, such as contacting with IL-2 or other exogenous cytokines. Cells produced in the first step by treatment with OKT3 and other cytokines are not the cells for re-

infusion into the patient, but are used to produce a supernatant that is used to treat cells, which then are exposed to Il-2 for expansion.

In contrast the instant methods, include the steps of differentiating leukocytes or mononuclear cells to become Th1 cells, and expanding the resulting Th1 cells to produce clinically relevant numbers of cells. Babbitt does not disclose or suggest the instantly claimed method. The description in col. 5, referenced by the Examiner refers to addition of cytokines to the T3CS supernatant, which is then used to contact cells to produce immunoreactive cells. Any description of Th1 cells, refers to production of Th1 cells in the first step of the method, which is used to produce the T3CS supernatant. Cells from the patient are then cultured in the supernatant, which contains a variety of cytokines. The resulting cells are the immunoreactive cells, referred to by Babbitt as EVA cells, **not** Th1 cells. As discussed above, the immunoreactive cells, are not Th1 cells. Therefore, Babbitt does not anticipate any of the instant claims.

THE REJECTION OF CLAIMS 22-29, 31-35, 155, 156, 158, 160, 162, 164-168 AND 170-172 UNDER 35 U.S.C. § 103(a)

Claims 22-28, 33-35, 155, 156, 158, 160, 162, 164, 166-168 and 170-172

Claims 22-28, 33-35, 155, 156, 158, 160, 162, 164, 166-168 and 170-172 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Babbitt *et al.* (U.S. Patent No. 5,766,920) in view of Cracauer because Babbitt allegedly teaches methods of producing Th1 cells and Cracauer teaches the use of a hollow fiber bioreactor for expanding cells. This rejection is respectfully traversed.

Relevant law

In order to set forth a prima facie case of obviousness under 35 U.S.C. §103, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to hav made the

changes that would produce the claimed product. See, e.g., Lind mnann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 1462, 221 USPQ 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch 23 USPQ 1780 (CAFC 1992); see, also, In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

The Claims

The claims are discussed above.

Analysis

As discussed above, Babbitt does not teach or suggest the instantly claimed method. Babbitt teaches a method in which mononuclear cells are removed from a patient and treated to produce a supernatant. The treatment can result in Th1 cells in the supernatant. The supernatant is collected and the Th1 cells are discarded. The supernatant is used to treat additional cells from a patient to produce EVA (*ex vivo* activated) cells that are immunoreactive cells, which as discussed above are **polyclonal T-cells that exist in a primed state of activation** that are:

> are multifunctional, i.e., possess an enhanced capacity to proliferate and produce cytokines ***upon further stimulation.*** (emphasis added)

These immunoreactive cells are stated by Babbitt to have a:

> low spontaneous level of immune function following processing, but are highly sensitized to respond to *low doses of second signals* up on further culture or *in vivo*.(emphasis added)

As discussed above, these resulting immunoreactive cells require further activation and include CTL cells and other cells; they not Th1 cells, nor does Babbitt teach or suggest that they are.

The disclosure to which the Examiner refers at col. 5 is not directed to producing Th1 cells for infusion into patients, but describes preparation of the T3CS supernatant that is used to produce the immunoreactive cells that are

reinfused into the patient. The cells expanded by Babbitt to 10^9 cells, are not Th1 cells, but are the immunoreactive (EVA) cells that result from culture in the T3CS supernatant. Therefore Babbitt fails to teach a method in which mononuclear cells or leukocytes are removed from a subject, treated to differentiate into Th1 cells, and then **these** cells, are treated in the absence of IL-2 or other exogenous cytokines to proliferate to produce compositions that contain high concentrations of Th1 cells. No where in Babbitt does it teach or suggest that Th1 cells are produced, expanded and reinfused into patients. The cells that are expanded are the EVA cells, not Th1 cells.

Cracauer, which teaches a bioreactor, does not cure the deficiencies in the teachings of Babbitt. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 22-29 31, 34, 155, 156, 158, 160, 162, 164, 167, 168 and 170-172

Claims 22-29 31, 34, 155, 156, 158, 160, 162, 164, 167, 168 and 170-172 are rejected under 35 U.S.C. §103(a) as being unpatentable over Babbitt in view of O'Garra *et al.* because O'Garra allegedly teaches that IL-4 antibody treatment of CD4+ cells favors development of Th1 cells so that it would have been obvious to one of ordinary skill in the art to have produced Th1 cells. This rejection is respectfully traversed.

Babbitt

The teachings of Babbitt are discussed above. As discussed above, Babbitt does not teach a method for production of clinically relevant numbers of Th1 cells, but rather teaches production of a supernatant, and use of the supernatant for production of immunoreactive cells, which are **not** Th1 cells nor does Babbitt teach, suggest or in any manner imply that such cells are Th1 cells. Babbitt states that they are **polyclonal T-cells that exist in a primed state of activation:**

O'Garra *et al.*

O'Garra *et al.* is directed to a study to assess the role of cytokines in determining T-lymphocyte function. O'Garra *et al.* teaches that encounter with a host antigen can result in either cell-mediated or humoral classes of immune response and that these responses are attributable to the heterogeneity of $CD4^+$ T cells. O'Garra *et al.* further teaches that mouse $CD4^+$ T cell clones can be divided into two predominant cytokine secretion profiles designated Th1 , which produce IL-2 and IFN-*y* and other factors that promote delayed-type hypersensitivity reactions, and Th2, which produce Il-4, Il-5 and IL-10. The subsets by virtue of the differing cytokine profiles cross-regulate immune responses. O'Garra *et al.* states (page 459, col. 1) that the ability to control the emerging Th cell phenotype [*in vivo*] following exposure to antigen offers the potential to induce a response appropriate for each pathogen. O'Garra presents the results of studies designed to elucidate the pathways by which each type of subset is induced. O'Garra *et al.* concludes (page 462):

> . . . The question of whether Th1 and Th2 cells all arise from a common precursor, possibly a Th0-type cell, and whether such populations are malleable or can be differentiated further, remains *an unresolved issue*, with important implications for the treatment of chronic disease. (emphasis added)

Thus O'Garra *et al.* does not teach that populations of Th1 and Th2 cells can be produced and does not teach how to produce such populations.

Therefore, O'Garra *et al.* concludes that it is not clear whether Th1 and Th2 phenotypes can be altered; it does not teach or suggest production of compositions of either predominantly Th1 or Th2 cells, and certainly does not provides any motivation to produce large number or high densities of cells of any type.

O'Garra *et al.* does not cure the deficiencies in the teachings of Babbitt. Therefore, the combination of teachings of Babbitt and O'Garra *et al.* does not result in the instantly claimed methods. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 34, 164 and 165

Claims 34, 164 and 165 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Babbitt *et al.* (U.S. Patent No. 5,766,920) in view of Cracauer as applied to claims 22-29 31, 34, 155, 156, 158, 160, 162, 164, 167, 168 and 170-172 and further in view of June *et al.* (WO 94/29436 or U.S. Patent No. 5,858,358) because June *et al.* teaches expansion of cells using anti CD3 and anti CD28 antibodies. This rejection is respectfully traversed.

As discussed above, the combination of Babbitt and O'Garra *et al.* does not result in any of the claimed methods because the combination does not suggest a method for producing clinically relevant numbers of Th1 cells nor the steps of the instantly claimed method. June *et al.* does not cure this deficiency.

As discussed in previous responses, June *et al.* teaches a method for inducing a population of T cells to proliferate by providing a first signal to activate the cells and then a second signal to stimulate proliferation. Activation of T cells is accomplished by contacting the cells with anti-CD3 antibody or anti-CD2 antibody. Proliferation is induced by contacting an activated population of T-cells with a second agent, such as anti-CD28 antibody, that stimulates an accessory molecule on the T cell surface.

Therefore, the combination of teachings of the cited references does not result in the instantly claimed methods. The Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: MICHEAL L. GRUENBERG

Serial No.: 08/700,565

Filed: July 25, 1996

For: AUTOLOGOUS IMMUNE CELL THERAPY: CELL COMPOSITIONS, METHODS AND APPLICATIONS TO TREATMENT OF HUMAN DISEASE

Art Unit: 1644

Examiner: Schwadron, R

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as First Class mail in an envelope addressed to:
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Washington, D.C. 20231, on this date.

08/13/01
Date

Stephanie Seidman

MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims 22-25, 28, 155, 157, 158 and 211 as follows:

22. (Thrice Amended) A method for generating clinically relevant cell numbers of [regulatory] Th1 [T lymphoid] cells, comprising:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the T lymphoid cells to alter their cytokine production profile by causing differentiation of the cells into Th1 [to regulatory] cells; and

(c) in the absence of Il-2, inducing cell proliferation and expanding the cells under conditions that produce at least about 10^{10} cells/liter of a homogeneous population of [regulatory] Th1 [T lymphoid] cells, wherein a homogeneous population of Th1 cells comprises greater than about 50% Th1 cells.

23. (Twice Amended) The method of claim 22, wherein the [T lymphoid] Th1 cells with altered cytokine profile are purified.

24. (Twice Amended) The method of claim 22, wherein the [T lymphoid] Th1 cells with altered cytokine profile are specific for a defined antigen.

25. (Amended) The method of claim 23, wherein the [T lymphoid] Th1 cells with altered cytokine profile are specific for a defined antigen.

28. (Twice Amended) The method of claim 22, wherein the [T lymphoid] Th1 cells are activated *ex vivo* in the presence of either or both interferon-γ and IL-2, whereby cells differentiate into Th1 cells.

155. (Twice amended) A method for generating clinically relevant numbers of [regulatory T] Th1 [lymphoid] cells for autologous cell therapy, comprising:

(a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

(b) treating the cells to induce differentiation of mononuclear cells into [regulatory] Th1 [T]cells[, wherein regulatory T cells are mononuclear cell that have the ability to control or direct an immune response, but do not act directly as effector cells in the response]; and

(c) contacting the resulting differentiated cells with two or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of [regulatory T] cells for autologous cell therapy are generated, wherein the contacting is effected in the absence of exogenous cytokines.

157. (Amended) The method of claim 155, wherein the treating and contacting steps [or treating or contacting step] occurs in the absence of exogenous cytokines.

158. (Amended) The method of claim 155, wherein the [regulatory] cells are specific for a selected antigen.

211. (Twice Amended) A method for generating immune cells for autologous cellular immunotherapy, comprising:

collecting leukocyte containing material from a mammal;

differentiating the leukocytes into Th1 [or Th2] cells; and

exposing the leukocyte containing material to two or more mitogenic monoclonal antibodies to induce *in vitro* cell proliferation sufficient for infusion into the mammal for use in an immunotherapy treatment, wherein the *in vitro* cell proliferation is produced without the use of exogenous interleukin-2.